

**Anatoxin-a, Cylindrospermopsin,
Adda Microcystins/Nodularins, & Saxitoxins Report***Project: City of Melbourne, Water Production*

Submitted to: Shaniese Alexander
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Sample Receipt Date: 13 July 19
Sample Condition: 4.2 °C upon arrival
Report# 190730_City of Melbourne, Water Production-POE
Date Prepared: 1 August 19
Prepared by: Kamil Cieslik

Table 1: Samples analyzed

<u>Sample Identification</u>	<u>Description/Site</u>	<u>Collection Date</u>	<u>Collection Time</u>
19-3167	POE-6055 Lake Washington Rd.	30 July 19	1230

Analytes: Anatoxin-a (ANTX-A), Cylindrospermopsin (CYN), Adda Microcystins/Nodularins (Adda MCs/NODs), Saxitoxin (STX/PSTs)

Analytical Techniques

Enzyme-Linked Immunosorbent Assay (ELISA)

Adda MCs/NODs

A microcystins/nodularins Adda ELISA (Abraxis) was utilized for the quantitative and sensitive congener-independent detection of Adda MCs/NODs (US EPA Method 546 & Ohio EPA DES 701.0). The current method reporting limit is 0.30 ng/mL (ppb) based on kit sensitivity, dilution factors, and initial demonstration of capability.

STX

A saxitoxin specific ELISA (Abraxis PN 52255B) was utilized for the detection and quantification of saxitoxin and related analogs (paralytic shellfish toxins – PSTs). The current method reporting limit is 0.05 ng/mL (ppb) based on kit sensitivity and dilution factors. Based on manufacture instructions, the STX ELISA is less cross-reactive to other PSTs and will likely underestimate total PSTs/Saxitoxins. Reported cross-reactivities are as follows: NEO (1.3%), dcSTX (29%), GTX2/3 (23%), GTX5 (23%), dcGTX2/3 (1.4%), dcNEO (0.6%) & GTX1/4 (<0.2%).

Liquid chromatography mass spectrometry/mass spectrometry (LC-MS/MS)

ANTX-A & CYN

A Waters XSelect HSS T3 2.1 x 150 mm, 3.5- μ m column was used in separation with mobile phases (methanol and water) containing acetic acid. The $[M+H]^+$ ion for ANTX-A (m/z 166) was fragmented and the product ions (m/z 91, 131, 149) were monitored. The $[M+H]^+$ ion for CYN (m/z 416) was fragmented and the product ions (m/z 194, 274, 336) were monitored. The $[M+H]^+$ ion for the internal standard [$^{15}N_5$]-Cylindrospermopsin (421 m/z) was fragmented and the product ion (341 m/z) was monitored. The $[M+H]^+$ ion for the internal standard [$^{13}C_4$]-Anatoxin-a (171 m/z) was fragmented and the product ion (153 m/z) was monitored. The internal standard method was utilized for all quantification.

Abbreviations

NA	Not Applicable	LFSM	Lab Fortified Sample Matrix
MDL	Method Detection Limit	LFSMD	Lab Fortified Sample Matrix Duplicate
MQL	Method Quantification Limit	LD	Lab Duplicate
ND	Not Detected above the MDL	IS	Internal Standard
Blank	Regent Water free from interferences	—	Not Analyzed
LFB	Lab Fortified Blank	MRL	Method Reporting Limit

Quality Control

Table 2: QA/QC samples prepared for analyses.

Analyte	Concentration (ng/mL)	Sample ID	QC Type	Return
MC-LR	1.0	19-3167	LFSM	86%
CYN	0.1	19-3168	LFSM	112%
[¹⁵ N ₅]-CYN	1.0	19-3167 & 19-3168	IS	87 ± 10%
ANTX-A	0.1	19-3168	LFSM	104%
[¹³ C ₄]-ANTX-A	1.0	19-3167 & 19-3168	IS	90 ± 6%
STX	0.2	19-3167	LFSM	60% ^N

Additional Quality Control/Quality Assurance checks included method blanks, LFBs, and standard curves.

Table 3: Adda MC-ELISA Quality Control Value Table

Date Analyzed:	1 August 19	Requirement	Pass/Fail
R² value:	0.997	≥0.98	PASS
%CV range STDs:	0.5-9.2%	≤15%	PASS
LFB (1ppb) Recovery:	91%	±40% True Value	PASS
%CV range LFB:	13.2%	<20%	PASS
Low CV (0.15 ppb) recovery:	113%	±50% True Value	PASS
LRB	<0.08	<0.08	PASS

Qualifier	Flag
CL	Analytical result is estimated due to ineffective quenching.
J	Analyte was positively identified; the associated numerical value is estimated.
PT	The reported result is estimated because the sample was not analyzed within required holding time.
B	Analytical result is estimated. Analyte was detected in associated reagent blank as well as the samples.
E	Analytical result is estimated. Values achieved were outside calibration range.
N	Spiked sample control was outside limits
T	The reported result is estimated because the sample exceeded temperature threshold when received

Summary of Results

Table 4: Summary of results in ng/mL

Sample ID	Adda MCs/NODs (ng/mL)	CYN (ng/mL)	ANTX-A (ng/mL)	STX (ng/mL)
19-3167	ND	ND	ND	ND
<i>MRL (ng/mL)</i>	<i>0.30</i>	<i>0.05</i>	<i>0.05</i>	<i>0.05</i>
<i>Analyst Initials</i>	<i>KC</i>	<i>MA</i>	<i>MA</i>	<i>KC</i>
<i>Date Analyzed</i>	<i>8/1/19</i>	<i>8/1/19</i>	<i>8/1/19</i>	<i>8/1/19</i>

Interpretations:

Adda MCs/NODs, CYN, ANTX-A, and STX were not detected in the submitted sample above the MRLs.

Submitted by:



Mark T. Aubel, Ph.D.

Date:

August 1, 2019

*The results in this report relate only to the samples listed above.
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